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Hydroxyproline Metabolism in Serum, Synovial Fluid and Synovial Membrane in Joint Diseases

by

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It is well known that hydroxyproline is a main component of collagen tissue in living body. The determination[1] of hydroxyproline, therefore, has been utilized for the measurement of changes in metabolism of collagen tissue.

In this paper, the authors attempt to determine, by the original method of Prockop[2], the hydroxyproline contained in serum, synovial fluid and synovial membrane taken from the patients who suffer from rheumatoid arthritis or osteoarthritis as they represent inflammatory and non-inflammatory diseases respectively.

MATERIALS AND METHODS

For this study, all cases of rheumatoid arthritis were classified into definite type and classical type by the criteria of the American Rheumatoid Association.

All cases of rheumatoid arthritis, 4 of 10 cases of osteoarthritis and a case of femoral tumor were taken from the patients in the ward of the Orthopedic Surgery in Iwate Medical University Hospital. 6 of 10 cases of osteoarthritis were from the out-patients.

Synovial-fluid and synovial-membrane were extracted from their knee joints.

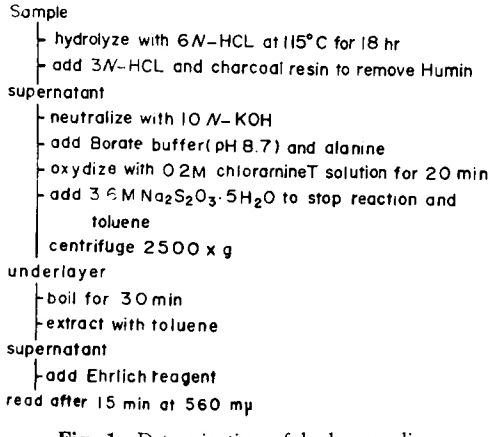

Fig. 1 Determination of hydroxyproline

The sample (serum and synovial fluid 2 ml, synovial membrane 50 mg) was hydrolyzed with 6 N-HCl at 115°C for 18 hours, and charcoal resin was added to it to remove Humin into hydrolysate. After centrifugation, the supernatant was neutralized with KOH, and borate buffer (pH 8 9) and alanine were added. It was, then, oxydised with 0. 2 M chloramine T solution for 20 minutes, and 3. 6 M-$Na_2S_2O_3 \cdot 5H_2O$ was added to it to stop its reaction. Further, after toluene was added, it was stirred hard. After the centrifugation, its underlayer was boiled for 30 minutes and extracted by toluene. Finally,

EHRLICH's reagent was added to the supernatant, and 15 minutes later, and read at 560 mμ.

RESULTS

The results obtained are shown in table 1-3. The mean hydroxyproline content in synovial fluid in rheumatoid arthritis was 4.72±0.56 μg/ml, and that in serum was 6.63

Table 1 HOP content in the blood and synovial fluid in the patient with rheumatoid arthritis

	sex	age	blood sedim. rate		ASLO	RA	CRP	hydroxyproline	
								synovial fluid	serum
			1hr.	2hr.					
G. K.	남	70	95	122	166	+	+	5.5 μg/ml	6.5 μg/ml
I. S.	여	54	13	39	100	±	+	3.8	7.2
H. K.	여	57	33	65	125	+	+3	4.8	6.3
S. H.	여	47	124	130	50	+	+5	4.4	6.6
T. K.	여	18	83	122	12	++	+	4.5	6.8
Y. S.	여	20	3	20	50	±	+	5.3	6.4
mean								4.72±0.56	6.63±0.30

Table 2 HOP content in the blood and synovial fluid in the patient with osteoarthritis

	sex	age	blood sedim. rate		ASLO	RA	CRP	hydroxyproline	
								synovial fluid	serum
			1hr	2hr					
Y. O.	남	53	1	6	50	−	−	3.6 μg/ml	5.8 μg/ml
M. I.	여	56	5	18	100	−	−	2.5	6.2
T. T.	남	55	5	17	12	−	−	2.9	7.3
K. U.	여	54	10	16	50	−	−	3.8	5.8
S. O.	여	55	9	21	125	−	−	2.5	6.1
M. K.	여	63	22	53	50	−	±	3.3	6.3
mean								3.20±0.51	6.25±0.27

±0.30 μg/ml. As for osteoarthritis mean hydroxyproline content obtained here was 3.20 ±0.51 μg/ml in synovial fluid and 6.25± 0.27 μg/ml in serum. As the results of both diseases are compared, no difference was found in hydroxyproline content in serum, but, in the cases of rheumatoid arthritis, mean hydroxyproline content in synovial fluid showed a slight increase as compared with that of osteoarthritis.

In rheumatoid arthritis, hydroxyproline content was found to be increased in serum more than in synovial fluid. Similar tendency was also observed in osteoarthritis. While

Table 3 HOP content in the synovial membrane in the patients with rheumatoid arthritis, osteoarthritis and tumor of femur

Rheumatoid arthritis	1	3.51mg/g
	2	2.74
	3	3.17
	4	3.48
	mean	3.20
Osteoarthritis	1	2.23
	2	1.88
	3	1.59
	4	1.70
	mean	1.85
Tumor of femor		2.60

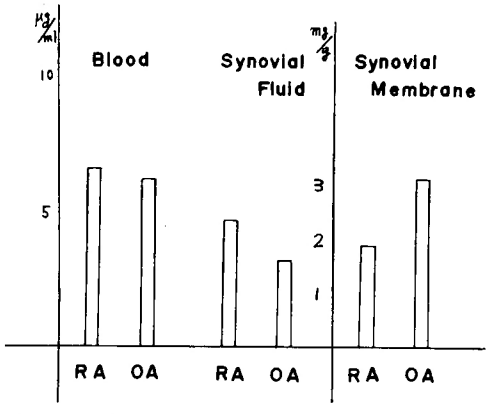

Fig. 2 HOP content in the Blood, synovial fluid and synovial membrane

mean hydroxyproline content in synovial membrane was 1.85 mg/g in rheumatoid arthritis, it was 3.20 mg/g in osteoarthritis, and 2.60 mg/g in femoral tumor.

DISCUSSION

Since the pathological findings of rheumatism are mainly observed in collagen tissue, the change of hydroxyproline content in serum and urine was admitted to be a good index of metabolic activity in collagen disease. On hydroxyproline in the patient with rheumatoid arthritis, MARY[3] (1952) first reported that its urinary excretion from the patient was 20 times as large as that of normal urine. Recently Le ROY[4] reported that in serum of most cases of rheumatoid arthritis hydroxyproline content increased more than that of normal adults. RUBEGUI[5] described that the excretion of free hydroxyproline from the patient with rheumatoid arthritis increased 2–5 times as large as that of normal adults.

SMITH[6] found that the urinary excretion of hydroxyproline is related closely with the condition of collagen disease and increased in the active period of rheumatism.

It was reported that hydroxyproline in the protein of whole body was not directly absorbed from the diet, but was mainly synthesized from proline in the body[7]. OKAZAKI[8] reported that, no change was found on proline in serum and synovial fluid taken from the patient with rheumatoid arthritis.

It is said that about 60% of collagen in human body originates from bone collagen.[9] Therefore, excretion of hydroxyproline in malignant bone tumor and congenital systemic bone disease was found to be increased remarkably in urine, because the increased soluble collagen in blood resulted from the destruction in bone tissue.

From the data obtained here, while no change was found on the hydroxyproline content in serum taken from the patient with rheumatoid arthritis or osteoarthritis, the hydroxyproline showed an increase in synovial fluid of rheumatoid arthritis more than that of osteoarthritis. On the contrary, it showed a decrease in synovial membrane of rheumatoid arthritis in comparison with that of osteoarthritis. It can be said, therefore, that there is a negative relation between the hydroxyproline contents in synovial fluid and synovial membrane with respect to both diseases. The results suggest that a part of collagen produced by dissimilation in synovial membrane dissolved into blood as a soluble collagen and was excreted as hydroxyproline metabolite, while a part of the rest was accumulated in joint cavity.

It appears that, in the cases of rheumatoid arthritis where bone and surrounding tissues are severely destroyed, hydroxyproline content decreases in synovial membrane, whereas it increases in synovial fluid and urine more than in osteoarthritis.

The hydoxyproline content in the case of rheumatoid arthritis increased more in serum than in synovial fluid and it showed a similar tendency also in osteoarthritis. The difference between its mean levels in serum and in synovial fluid was greater in rheumatoid

arthritis than in osteoarthritis.

On the other hand, similar results were observed on the contents of vitamine B_2[10], tyrosine and tryptophan[11] in our experiment which is now carried on in our laboratory. It is presumed from these results that the permeability of synovial membrane may be increased more in the case of rheumatoid arthritis than in osteoarthritis. SUGIYAMA[10] described, however, that the rise of permeability had been ascertained by riboflavin loading test in joint cavity in the case of rheumatoid arthritis. From these points of view, permeability of synovial membrane plays an important role in the maintenance of metabolism in articular cavity, but, it must be, of course, considered that there is also independent metabolism in the articular cavity which is quite free from the influence of permeability.

It is reported that steroid and vitamine C have great effect upon urinary excretion of hydroxyproline. ISHIKAWA[12] described that a group of patients, which was at the stage I of rheumatoid arthritis, showed a decrease of hydroxyproline excretion after administration of vitamine C, but no change was shown after steroid admistration. Another group at the stage II-IV showed an increase of hydroxyproline excretion after administration of steroid, but no change was shown after vitamine C was given. The discrepancies shown in urinary hydroxyproline excretion after the administration of vitamine C and after that of steroid are due to the different effects of the chemicals on turnover of collagen tissue.

According to ABE[13], in serum of rheumatoid arthritis, vitamine C content was lower than that of healthy subjects. REGBY[14] described that collagen degenerated in the state of inflammation and its solubility increased. It is presumed, therefore, that the decrease of hydroxyproline excretion in urine after the administration of vitamine C may be ascribed to the increased assimilation and the decreased dissimilation of collagen, which has once denaturated by inflammation, because vitamine C content restored to its normal level in serum.

We are now going to look for a relation of vitamine C and steroid to hydroxyproline metabolism in synovial fluid in the cases of joint disease.

SUMMARY

Hydroxyproline in serum, synovial fluid and synovial membrane taken from patients with rheumatoid arthritis or osteoarthritis was determined by the original method of Prockop. In the cases of rheumatoid arthritis, hydroxyproline showed a slight increase in synovial fluid more than that of osteoarthritis, whereas it showed a decrease in synovial membrane in the cases of rheumatoid arthritis as compared with that of osteoarthritis. No difference was found in serum with respect of both diseases.

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和文抄録

関節疾患おける Hydroxyproline 代謝について

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炎症性及び非炎症性疾患を代表する関節リウマチ及び変形性関節症における血液，関節液及び滑膜組織中の hydroxyproline 含有量を Prockop 原法に準じて測定した．

関節リウマチ患者の関節液中の hydroxyproline 含有量は平均4.72±0.56 μg/ml であり，血液中の hydroxyproline 含有量は平均 6.63±0.30 μg/ml であつた．これに対して変形性関節症の関節液中の hydroxyproline 含有量は平均3.20±0.51 μg/ml であり，血液中の hydroxyproline 含有量は平均6.25±0.27 μg/ml であつた．両疾患の成績を比較してみると，血液中の hydroxyprolineは関節リウマチがやや高値を示したが有意差は認められなかつた．一方関節液中の hydroxyproline は関節リウマチが変形性関節症に比較して，かなりの高値を示した．また関節リウマチの血液及び関節液中の hydroxyproline 値を比較してみると，関節液よりも血液中の hydroxyproline が高値を示した．変形性関節症においても同様の成績を得た．

また滑膜組織中の hydroxyproline は関節リウマチが1.85mg/gと低値を示し，変形性関節症では 3.20mg/g とかなりの高値を得た．

したがつて血液中の hydroxyproline 値は両疾患とも有意差を認めなかつたが，関節液中の hydroxyproline 値は関節リウマチが変形性関節症よりも高値を示し，滑膜組織では逆に変形性関節症が約2倍の高値を示した．

この成績から，滑膜組織における異化作用により生じた collagen は一部が可溶性 collagen として血液中に流出し，分解されて尿中に hydroxyproline として排泄され，また残りの一部は不可溶性 collagen として関節液中に遊離し蓄積されるものと考えられる．そのために組織破壊の著しい関節リウマチにおいては，滑膜自体の hydroxyproline 含有量が減少し，尿中及び関節液中では増加するものと考えられ，また組織破壊の少ない変形性関節症では，その逆の結果が得られるものと考える．

両疾患の血液中及び関節液中の hydroxyproline の差を比較してみると，関節リウマチでは少なく，変形性関節症では大であつた．これは関節リウマチの滑膜の透過性増大の関与も否定出来ないが，関節内においてビタミンやアミノ酸等の特有な pattern を示す代謝が存在するものと考えている．